Inhibition of povidone-iodine's bactericidal activity by common organic substances: An experimental study

Jose L. Zamora, M.D., Margaret F. Price, Ph.D., Peter Chuang, M.S., and Layne O. Gentry, M.D., *Houston, Tex.*

An in vitro study demonstrated that some organic substances commonly present in the surgical field inhibit the bactericidal activity of dilute povidone-iodine solutions. The degree of inhibition was inversely proportional to the concentration of the povidone-iodine solutions and it was greatest by blood, followed by pus, fat, and glove powder. The pattern of bacterial kill was virtually identical for all the strains tested. The most likely explanation for this phenomenon is that iodine is bound by the organic substances, decreasing the iodine available for bacterial kill. To obtain an optimal benefit, we suggest eliminating these substances from the operative site or wound when possible before the use of povidone-iodine solution.

From the Department of Surgery and the Departments of Medicine and Microbiology, Section of Infectious Diseases, Baylor College of Medicine, Houston, Tex.

POVIDONE-IODINE (PVP-I), an antiseptic introduced by Shelanski and Shelanski[8] in 1953,* has become one of the most widely used products in the sphere of surgery. The main applications of this compound include hand degerming, operative site preparation, and traumatic and burn wound antisepsis. The bactericidal activity of PVP-I is exerted by free iodine that is released from it and bound to bacteria in the antiseptic-microbe interaction.[1, 6] Clinical and laboratory studies have suggested that iodine from PVP-I solutions is bound by organic substances (necrotic tissue, hemoglobin) that modify or decrease its antiseptic activity.[4, 6]

We performed an investigation to determine if the antibacterial activity of PVP-I solution is affected by exposing the antiseptic to organic substances commonly found in the surgical field and if varying the concentration of the PVP-I solution or using different strains of bacteria has any bearing on the degree of inactivation of PVP-I solutions.

The organic substances used in the study were glove

powder (corn starch), pus and fat homogenates, and blood; these substances were chosen because they are commonly found in the surgical environment. The concentrations of the PVP-I solution used are all compatible with clinical use and ranged from 0.001% to 5%. The strains of bacteria tested include some of the most common agents responsible for surgical infections.

MATERIAL AND METHODS

Povidone-iodine. Ten percent PVP-I stock solution (Pharmadyne; Sherwood Laboratories, Inc.,) was used to prepare the final concentrations by diluting with the organic substance to obtain 5%, 1%, 0.1%, and 0.001% PVP-I solutions.

Organic substances

Buffer. Citrate phosphate buffer pH 7 was prepared according to standard tables and sterilized by passage through a 0.45 millipore filter (Nalge Co., Rochester, N.Y.).

Glove powder solution. A suspension of absorbable dusting powder (U.S.P.) (cross-linked purified corn starch) was obtained by a modification of the "glove juice" method. After a sterile technique, 10 ml of sterile buffer solution was poured into a freshly opened prepowdered surgical glove (Pharmaseal Laboratories, Glendale, Calif.), the glove was squeezed for 5 minutes, and the glove powder-buffer solution was retrieved.
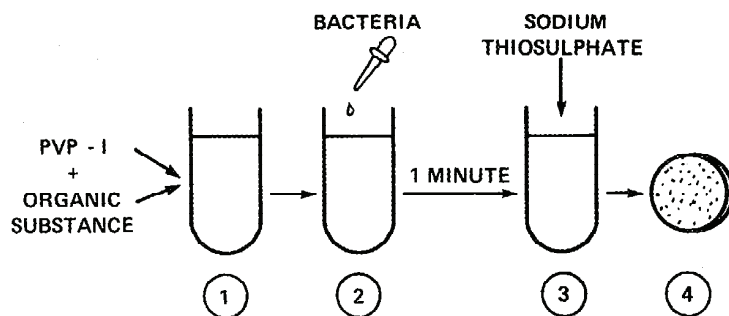
Fig. 1. Test Procedure. (1) Equal amounts of PVP-I solution and the organic substance are mixed, (2) 10 μl of bacterial suspension is added and agitated for 1 minute, (3) the remaining free iodine is inactivated with sodium thiosulphate to a final concentration of 0.025M solution, and (4) the mixture is plated on soy agar and the plates are incubated at 35° C and the colonies counted. (See detailed list of substances and solutions in the Materials and Methods section.)

Human samples. The samples of fat and pus were prepared to simulate volume per volume the approximate concentration of these substances present in a surgical wound.

The whole blood was used undiluted. All human samples were obtained from subjects without any previous antibiotic therapy.

Fat homogenate. A human fat suspension was obtained from the subcutaneous tissue of the abdominal wall and the thigh during the closure of the incisions in a "clean case." Meticulous hemostasis was performed, sterile normal saline solution was poured to fill the wounds, and the subcutaneous tissue was manually washed for 5 minutes, carefully saving the resulting suspension of saline plus fat and fat globules. A sterile cup container was submerged in the wound to remove the fluid while the subcutaneous tissue was scraped lightly with the edge of the container. The solid: liquid ratio of this suspension was determined volume per volume by centrifugation; it was 14% fat and 86% diluent. The saline was removed and the fat resuspended to a 14% suspension in sterile buffer citrate, homogenized by sonication, and agitated constantly during the process of mixing with the PVP-I solutions to avoid formation of supernatant. A control culture on blood agar verified the sterility of the fat samples.

Pus homogenate. Human pus aspirated from a perirectal abscess before a surgical drainage procedure was pooled and sterilized by treatment with ethylene oxide gas. The sterile pus was then diluted to give a final 14% v/v in sterile citrate buffer as in the fat suspension.

Blood. Whole blood was collected from a healthy donor, citrated, and kept sterile until use.

Bacterial suspension. To standardize the number of each of the bacterial species used, a calibration curve of optical density versus cell number (colony-forming units per milliliter) was prepared. A suspension from a 24-hour culture was then prepared in sterile saline solution to give the optical density equivalent to 10 colony-forming units of the appropriate bacteria per ml. Ten microliters of this solution was then used to inoculate the tubes containing the range of concentrations of PVP-1. All the bacteria used in this study were obtained from the American Type Culture Collection (ATCC) with this exception of two isolates of *Pseudomonas cepacia,* which were supplied by Donald Craven, M.D., of the Boston City Hospital. The strains tested were the following: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter anitratus* ATCC 19606, *P. cepacia* strains 4 and 6 (from the Maxwell Simon Laboratory for Infectious Diseases, Boston City Hospital, Boston, Mass.), *Staphylococcus aureus* ATCC 25923, *Serratia marcescens* ATCC 8100, and *Streptococcus faecalis* ATCC 29212.

Test procedure. Aerobic and anaerobic cultures of the organic substances obtained before the experiment was started yielded no growth.

Ten percent PVP-I was diluted in each of the organic substances to yield final concentrations of 5%, 1%, 0.1%, 0.01%, and 0.001% of PVP-I. Two control tubes consisting of 0% PVP-I were included in each batch, and 10 μl of the bacterial suspension to be tested was added to both control tubes and sodium thiosulphate to one control. We demonstrated that sodium thiosulphate has no antibacterial effect. Aliquots of each tube or serial dilution sample were transferred to tryptic soy agar (Fig. 1). These plates were incubated at 35° C for 24 to 48 hours and the number of colonies per plate were counted. To determine percentage of growth, one to three trials per bacteria per dilution

Table I. PVP-I plus citrate phosphate buffer control experiment; the buffer was used as diluent for the glove powder, fat, and pus homogenates; percent of bacterial growth as compared with the control tubes

Organism	Control 0 (%)	Percent PVP-I solution 0.001	0.01	0.1	1.0	5.0
E. coli	100	84				
P. aeruginosa	100	84				
A. anitratus	100	≧100				
P. cepacia4	100	91		No growth		
P. cepacia6	100	≧100		All organisms		
S. aureus	100	≧100				
S. marcescens	100	91				
S. faecalis	100	75				
All	100	90 ± 8				

Table II. PVP-I plus glove power (corn starch) solution, percent of bacterial growth as compared with the control tubes

Organism	Control 0 (%)	Percent PVP-I solution 0.001	0.01	0.1	1.0	5.0
E. coli	100	≧100	≧100			
P. aeruginosa	100	≧100	30			
A. anitratus	100	76	86			
P. cepacia4	100	82	87	No growth		
P. cepacia6	100	77	77	All organisms		
S. aureus	100	90	65			
S. marcescens	100	≧100	88			
S. faecalis	100	99	98			

Table III. PVP-I plus fat homogenate; percent of bacterial growth as compared with the control tubes

Organism	Control 0 (%)	Percent PVP-I solution 0.001	0.01	0.1	1.0	5.0
E. coli	100	≧100	≧100			
P. aeruginosa	100	≧100	≧100			
A. anitratus	100	98	≧100			
P. cepacia4	100	93	96	No growth		
P. cepacia6	100	84	83	All organisms		
S. aureus	100	91	≧100			
S. marcescens	100	90	83			
S. faecalis	100	≧100	96	GI		

Legend: GI, Growth inhibited but not prevented.

Table IV. PVP-I plus pus homogenate; percent of bacterial growth as compared with the control tubes

Organism	Control 0 (%)	Percent PVP-I solution 0.001	0.01	0.1	1.0	5.0
E. coli	100	NT	90	≧100		
Ps. aeruginosa	100	≧100	95	≧100		
A. anitratus	100	NT	98	94		
P. cepacia4	100	99	96	93		
P. cepacia6	100	≧100	>100	≧100		
S. aereus	100	≧100	99	98		
S. marcescens	100	99	≧100	≧100		
S. faecalis	100	94	≧100	≧100		

Legend: NT, Not tested.

were performed depending on the overall pattern of growth and consistency of the results obtained.

RESULTS

The results of the experiment are shown in Tables I through V. The buffer solution was used as diluent of the other substances; therefore a control experiment was performed to test the effect of the buffer solution alone (Table I). The organic substances inhibit the bactericidal activity of PVP-I solution. The concentration that achieved a virtual total kill of all bacteria was considered the cutoff point for each given organic substance. This was 0.1% for glove powder, 0.1% for fat homogenate, 1% for pus homogenate, and 5% for blood (Tables II through V). The kill rates of the PVP-I solutions for the various bacteria were almost identical (Table V).

The effect of the contact with the substances was distinct; the resulting cultures yielded either near-total kill or greater that 80% growth for each solution.

The results of bacterial growth were compared with those in the two control tubes included in each experiment and expressed as pecentage of growth.

There were three instances in which the bacterial growth was inhibited but not prevented (Tables III and V). This was interpreted as a bacteriostatic but not bactericidal effect.

DISCUSSION

Recent laboratory reports have shown that, because of their higher "free iodine" content, dilute PVP-I solutions (0.01%, 0.1%, and 1%) achieve greater kill rates at 15 seconds than do more concentrated ones (5% or 10%); however, at 60 seconds the kill rates are similar.[1] This reflects the ability of the PVP-I complex to act as a reservoir of free iodine and to continue to release it from the bound to the free form until a balance is reached and the iodine-binding sites are
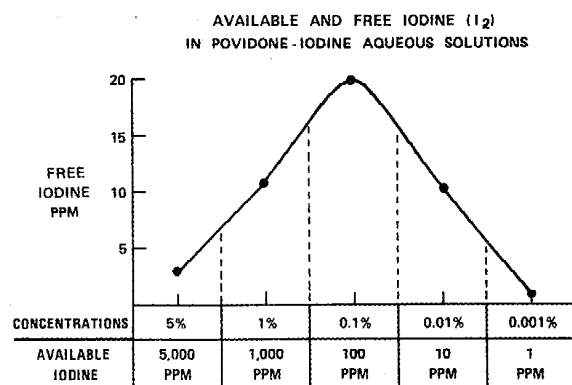
AVAILABLE AND FREE IODINE (I₂)
IN POVIDONE-IODINE AQUEOUS SOLUTIONS

CONCENTRATIONS	5%	1%	0.1%	0.01%	0.001%
AVAILABLE IODINE	5,000 PPM	1,000 PPM	100 PPM	10 PPM	1 PPM

Fig. 2. Distribution of free and available iodine in aqueous solutions of PVP-I.

Table V. PVP-I plus blood; percent of bacterial growth as compared with the control tubes

Organism	Control 0 (%)	Percent PVP-I solution 0.001	0.01	0.1	1.0	5.0
E. coli	100	96	≧100	96	≧100	
P. aeruginosa	100	≧100	≧100	≧100	80	
A. anitratus	100	≧100	≧100	78	72	
P. cepacia¹	100	≧100	86	≧100	≧100	
P. cepacia⁶	100	89	87	94	97	
S. aureus	100	≧100	≧100	97	≧100	GI
S. marcescens	100	85	77	81	81	
S. faecalis	100	75	≧100	≧100	≧100	GI

Legend: GI, growth inhibited but not prevented.

saturated (i.e., all bacteria are killed) or the iodine content is exhausted. In this regard dilute solutions are at a disadvantage because they have a smaller iodine reservoir and therefore the capacity to kill fewer bacteria. In our experiment the contact with organic soils before the bacterial challenge extracted iodine from the PVP-I solution, with a decrease in antibacterial activity inversely proportional to the concentration of the solution. The more concentrated ones were able to saturate the binding sites in the organic matter and retain enough iodine to achieve near-total kill.

The distribution of "available" and "free iodine" in PVP-I solutions of concentrations equal to the ones we tested has been described in detail by Winicov and Winicov[10] and Horn and Ditter.[5] According to their findings, the available iodine has a linear correlation with the concentration of the solution while the free iodine follows a bell-shaped curve with the peak at approximately 0.1% solution (Fig. 2).

Testing of human samples in concentrations similar to those found in surgical wounds demonstrated that the inhibiting effect was greatest by blood, followed by pus and fat, most likely reflecting their content of binding sites for iodine. However, taking into consideration the limitations of in vitro studies and the many variables present in the clinical setting, we did not attempt to determine the exact iodine-binding capacity of the substances but simply do describe qualitatively the overall pattern of inhibition.

The kill rates were essentially identical for all the bacterial strains. The strains of *P. cepacia* used were obtained from laboratory stock that was begun with isolates from a nosocomial infection of PVP-I solutions reported in the literature where the solution was intrinsically contaminated[2]; these strains failed to show resistance to PVP-I solution in our tests. At the first concentration that achieved total kill, there were three instances in which abnormal colonies were noted and bacterial growth was inhibited but not prevented; the strain of bacteria was different in all three cases (Tables III and V). This growth may reflect survival in subbactericidal or bacteriostatic concentrations of iodine, a phenomenon that has been reported previously.[3,7]

The findings of our study may explain at least partially the confusing results obtained in laboratory animal investigations that have tested PVP-I for prophylaxis of wound infection.[11] The results published in the literature are contradictory; most of the models have used inoculation of the peritoneal cavity or subcutaneous tissue with bacteria to achieve an infection followed by treatment with PVP-I topical application. In this experimental setting variable amounts of organic solid (pus, fat, and blood) are present in the wound and interact with PVP-I, therefore the actual amount of iodine interacting with bacteria is unpredictable; it is likely that a similar phenomenon may occur clinically during the practice of peritoneal and wound irrigations as recommended by some authors.[9]

The PVP-I solutions we tested are of lower concentration than the 10% solution commonly used for operative site preparation. This PVP-I paint contains enough iodine to achieve degerming and to allow a dry layer of antiseptic to remain on the skin; however, its antibacterial effect cannot persist if there has been overflow of blood or blood fluid onto the PVP-I painted areas and the typical PVP-I color has disappeared. In contaminated and dirty cases this overflow of organic soil, in addition to inactivating the skin preparation, may also seed the area with pathogenic bacteria and constitute focus of future contamination of dressings, drains, intravenous lines, monitoring catheters, etc. It

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seems recommendable at the end of a contaminated or dirty case to cleanse with normal saline solution all areas covered by overflow from the operative wound and apply a coat of 10% solution to inhibit the growth of pathogenic bacteria.

The message of our article as it applies to daily surgical practice is that blood, pus, and fat inhibit the bactericidal activity of PVP-I solution. To obtain an optimal benefit, we suggest removing these substances when possible from the operative site of wound before applying this antiseptic when its use is indicated.

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Inhibicion de la actividad bactericida de yodo-povidone for substancias organicas comunes

En un estudio in-vitro se demostró que algunas de las substancias orgánicas habitualmente presentes en el campo operatorio, inhiben la actividad bactericida de la soluciones diluidas de yodo-povidona. El grado de neutralización de la actividad estuvo en relación inversamente proporcional a las concentraciones de PVP-I. El grado mayor de inhibición fue producido por la sangre, seguido de pus, grasa y talco de los guantes. El patrón de destrucción bacteriana fue prácticamente igual para todas las clases de bacterias estudiadas. La explicación mas factible de este fenómeno, es que el yodo se enlaza con las substancias orgánicas disminuyendo asi la cantidad de yodo disponible para destruir bacterias. Con el objeto de obtener un máximo beneficio, sugerimos eliminar dentro de lo posible estas substancias de la herida o campo operatorio antes de la utilización de las soluciones de yodo-povidone.